PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB2005/001463	15.04.2005	15.04.2004

International Patent Classification (IPC) or both national classification and IPC
C07K16/44, A61K47/48, G01N33/92, G01N33/68

Applicant
ATHERA BIOTECHNOLOGIES AB

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 9

because:

☒ the said international application, or the said claims Nos. 9 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9
	No:	Claims	10-16
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-16
Industrial applicability (IA)	Yes:	Claims	1-8,10-16
	No:	Claims	9

2. Citations and explanations

   see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

   and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

   see form 210

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III.

Claim 9 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of this claim (Article 34(4)(a)(I) PCT).

Re Item IV.

The separate inventions/groups of inventions are:

No.	Claims	
1.	1-3 and 9 in part, and 4-8	Use of a phosphorylcholine conjugate in the treatment of atherosclerosis or related disease, and corresponding method of prophylactic or therapeutic treatment.
2.	1-3 and 9 in part	Use of an antibody specific for a phosphorylcholine conjugate in the treatment of atherosclerosis or related disease, and corresponding method of prophylactic or therapeutic treatment.
3.	10-15	Method of diagnosing the presence or absence of IgM or IgG antibodies as defined in these claims.
4.	16	Use of a phosphorylcholine conjugate for assessing a patient's risk of developing or progression of ischemic cardiovascular disease as defined in this claim.

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

The three problems underlying the present application are to provide a therapeutic or prophylactic use or method for atherosclerosis (claims 1-9), to provide a method for diagnosing the presence or absence of IgM or IgG antibodies (claims 10-15), and a use for assessing a patient's risk of developing or progression of ischemic cardiovascular disease (claim 16). As solution to each of these problems, an immunogenic conjugate of phosphorylcholine (PC) is proposed. To the first problem, an anti-PC antibody is further

proposed (claims 1-3, 9).
The common technical feature linking these different subjects is the relationship between anti-PC immune response or anti-PC antibodies and the reduction of atherosclerosis risk. This link has, however, already been described in the prior art.

Database Dissertation Abstracts [Online], ProQuest Info&Learning; 2002, Binder, Christoph Johannes: "Defining innate and adaptive immune mechanisms in the atheroprotective effect of immunization with oxidized low-density lipoproteins" retrieved from DIALOG accession no. 01907366 Database accession no. AADAA-I3064459 shows that anti-PC antibody T15 = EO6 protect against S. Pneumoniae and against atherogenesis as well.
Nature Medicine, vol. 9, no. 6, June 2003, pages 736-743, ISSN: 1078-8956 teaches that pneumococcal vaccination decreases atherosclerotic lesion formation, due to the fact, that the antibodies elicited against pneumococcus also target these lesions.
The authors of **Nature Medicine, vol. 9, no. 6, 1 June 2003 (2003-06-01), pages 641-642, ISSN: 1078-8956** demonstrate, that increasing the level of anti-PC antibody T15 reduces the development of atherosclerosis in mice. These data suggest that vaccines which increase the level of T15 can protect against atherosclerosis.
This suggestion is also found in the document **Nature Medicine, vol. 8, no. 11, 1 November 2002 (2002-11-01), pages 1218-1226, ISSN: 1078-8956**.

These documents thus anticipate the technical feature linking the different subjects contained in the present application. Therefore, this technical feature can no longer serve as special technical feature in the sense of Rule 13 PCT, linking the different subjects together.
Since there is no other technical feature, that could fulfil the role of special technical feature in the sense of Rule 13 PCT, the present application lacks unity of invention, containing the subject-matters as listed.
In this context it is pointed out, that the distinction between an antibody against phosphorylcholine and an antibody against phosphorylcholine **conjugates** (as defined by exact wording in the claims) is artificial. Indeed, for the production of antibodies, it is standard practice to use a conjugate of a large molecule and the hapten to elicit an immune response. This standard practice is reflected for phosphorylcholine in **WO 99/33522 A**, **US 5 455 032 A** or **EP 0 466 505 A**.

In principle, each of the compounds mentioned in the claims represents a different invention. However, in order to reduce the number of subjects as much as possible, the compounds have been regrouped according to structural similarities, and to the different problems to be solved.
As the applicant has paid a search fee for all inventions, all were searched.

Re Item V.

Reference is made to the following documents:

D1 : Database Dissertation Abstracts [Online] ProQuest Info&;Learning; 2002
Binder, Christoph Johannes: "Defining innate and adaptive immune mechanisms in the atheroprotective effect of immunization with oxidized low-density lipoproteins"
retrieved from DIALOG accession no. 01907366
Database accession no. AADAA-I3064459

D2 : Binder, Christoph J. ET AL: "Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between Streptococcus pneumoniae and oxidized LDL"
Nature Medicine, Vol. 9, no. 6, June 2003 (2003-06), pages 736-743, XP002355525
ISSN: 1078-8956

D3 : Rose N ET AL: "Autoimmunity: Busting the atherosclerotic plaque"
Nature Medicine, vol. 9, no. 6, 1 June 2003 (2003-06-01), pages 641-642, XP002355526 ISSN: 1078-8956

D4 : Binder C J ET AL: "Innate and acquired immunity in atherogenesis"
Nature Medicine, vol. 8, no. 11, 1 November 2002 (2002-11-01), pages 1218-1226, XP002355527 ISSN: 1078-8956

D5 : Shaw P X ET AL: "The autoreactivity of anti-phosphorylcholine antibodies for atherosclerosis-associated neo-antigens and apoptotic cells"
JOURNAL OF IMMUNOLOGY 15 JUN 2003 UNITED STATES, vol. 170, no. 12, 15 June 2003 (2003-06-15), pages 6151-6157, XP002355528 ISSN: 0022-1767

D6 : Binder Christoph J ET AL: "Molecular mimicry between epitopes of oxidized LDL and Streptococcus pneumoniae"
ABSTRACTS FROM AMERICAN HEART ASSOCIATION SCIENTIFIC SESSIONS 2000, [Online] 12 November 2000 (2000-11-12), XP002355529 NEW ORLEANS,

LOUISIANA, US, Abstract ID: 108867 Retrieved from the Internet: URL:http://aha.agora.com/abstractviewer>; [retrieved on 2005-11-10]

D7 : Purkall D ET AL: "Opsonization of Actinobacillus actinomycetemcomitans by immunoglobulin G antibody reactive with phosphorylcholine" Infection and Immunity, vol. 70, no. 11, 2002, pages 6485-6488, XP002355530 ISSN: 0019-9567

D8 : WO 99/33522 A (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM; SCHROIT, ALAN, J) 8 July 1999 (1999-07-08)

D9 : US 5 455 032 A (KENNY ET AL) 3 October 1995 (1995-10-03)

D10 : Shoji Tetsuo ET AL: "Inverse relationship between circulating oxidized low density lipoprotein (oxLDL) and anti-oxLDL antibody levels in healthy subjects" Atherosclerosis, Vol. 148, no. 1, January 2000 (2000-01), pages 171-177, XP002355531 ISSN: 0021-9150

D11 : WO 01/32070 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA; WITZTUM, JOSEPH; TSIMIKAS) 10 May 2001 (2001-05-10)

D12 : WO 02/080954 A (FORSKARPATENT I SYD) 17 October 2002 (2002-10-17)

D13 : WO 01/68119 A (KAROLINSKA INNOVATIONS AB; HANSSON, GOERAN, K; STEMME, STEN; NICOLETTI) 20 September 2001 (2001-09-20)

D14 : WO 90/12632 A (THE UNITED STATES OF AMERICA, REPRESENTED BY THE S) 1 November 1990 (1990-11-01)

D15 : KOH-ZOH KAMEYAMA ET AL: "CONVENIENT PLASMID VECTORS FOR CONSTRUCTION OF CHIMERIC MOUSE/HUMAN ANTIBODIES" FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, Vol. 244, no. 2, 27 February 1989 (1989-02-27), pages 301-306, XP000007812 ISSN: 0014-5793

D16 : EP 0 466 505 A (FUJITA HEALTH UNIVERSITY; TAKARA SHUZO CO. LTD) 15 January 1992 (1992-01-15)

D17 : WO 94/14454 A (ENTREMED, INC) 7 July 1994 (1994-07-07)

D18 : US 5 955 584 A (DITLOW ET AL) 21 September 1999 (1999-09-21)

D19 : KEARNEY JOHN F: "Immune recognition of OxLDL in atherosclerosis" JOURNAL OF CLINICAL INVESTIGATION, Vol. 105, no. 12, June 2000 (2000-06), pages 1683-1685, XP002367018 ISSN: 0021-9738

D20 : CHYU KUANG-YUH et al: "Changes in innate and adaptive humoral immune responses and indices of atherosclerosis in aging." Journal of the American College of Cardiology, vol. 43, no. 5, Supplement A, 3

March 2004 (2004-03-03), page 499A, abstract no. 1122-173, XP002367019 & 53rd Annual Scientific Session of the American College of Cardiology; New Orleans, LA, USA; March 07-10, 2004 ISSN: 0735-1097

D21 : WO 93/18161 A (THE ROCKEFELLER UNIVERSITY) 16 September 1993 (1993-09-16)

D22 : US 5 475 100 A (HASHINO ET AL) 12 December 1995 (1995-12-12)

D23 : SHAW PETER X ET AL: "Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity" JOURNAL OF CLINICAL INVESTIGATION, Vol. 105, no. 12, June 2000 (2000-06), pages 1731-1740, XP002204419 ISSN: 0021-9738

2 Invention 1

Document D1 discloses that anti-PC antibody T15 = EO6 protects against S. Pneumoniae and inhibits atherogenesis.
Document D2 discloses the anti-atherogenic effect of pneumococcal immunisation. The underlying mechanism is the fact, that in both cases the antibody is specific for phosphorylcholine.
Document D3 discloses that, "contrary to the more well-accepted notion that autoimmunity associated with atherosclerosis leads to disease, Binder, Hörkkö et al.3, in this issue, propose that autoimmunity can be protective. The authors provide evidence that a natural autoantibody to oxidized LDL (oxLDL), called T15, does not produce atherosclerosis in a mouse model, but rather decreases the extent of the disease. The data suggest that vaccines that boost T15 levels might protect against atherosclerosis".
Document D4 mentions that "an increased titer of EO6 antibodies would be expected to be protective, as these antibodies potently block macrophage uptake of oxLDL".
Document D5 discloses that the anti-PC antibody also reacts with antigens linked to atherosclerosis.
Document D6 suggests the link between vaccination and the reduction of atherogenesis.
Document D7 discloses the antimicrobial effect of anti-PC antibody.
Document D8 discloses the conjugates of PC with different proteins, which elicit an anti-PC antibody response in vivo.
Document D9 discloses the conjugates of PC with different proteins, which elicit an anti-

PC antibody response in vivo. The detection of these antibodies is given the last example, with the results in table 2.

Documents D1 to D 6 each suggest that vaccines which increase antibodies like EO6 protect against atherosclerosis. The present application does not seem more than the mere following of this explicit suggestion, and therefore does not meet the requirements of Article 33.3 PCT for inventive step.

The applicant might argue that the skilled person would not find in these documents the suggestion of how to elicit such an immune response. In that case, the problem underlying the present application would be to provide a new way of eliciting an anti-PC immune response. However, each of documents D7 to D9 describe, that conjugation of phosphorylcholine to a large peptide like BSA elicits such an immune response. Therefore, the skilled person, starting from any of D1 to D6 as closest prior art, would certainly apply the techniques described in any of D7 to D9, thus arriving at the presently claimed invention. For this reason, too, the present application does not meet the requirements of Article 33.3 PCT for inventive step.

For the assessment of the present claims 9 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognise as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Invention 2

Document D10 discloses the inverse relationship between circulating oxidized low density lipoprotein (OxLDL) and anti-OxLDL antibody levels in healthy subjects. Invention 2 of the present application can be distinguished from this prior art by the fact, that these findings are applied in the therapeutic treatment of atherosclerosis, by using such an antibody.

However, in the prior art, several antibodies are already known from the prior art for the

treatment of atherosclerosis.
Document D11 discloses antibody IK17. This antibody detects OxLDL; a marker for atherosclerosis. Hence it is proposed for targeting atherosclerotic drugs.
Also, both documents D12 and D13 disclose the use of a different antigen to elicit anti-atherosclerotic immune response.
Document D15 discloses the use of a hybridoma for producing an anti-phosphorylcholine antibody. This antibody has retained its specificity for the PC-OVA conjugate.
Document D17 discloses a sterol-based vaccine against atherosclerosis.
Perhaps more specifically, document D16 discloses the production of antibodies specific for PC-KLH, as demonstrated by example 4.
The skilled person, whishing to solve the problem of providing a treatment for atherosclerosis, knowing from D10 that increased anti-OxLDL antibodies reduce the risk of atherosclerosis, would certainly apply the teachings of any of D11-D13 or D15-D17, thus arriving at the treatment of atherosclerosis using an anti-phosphorylcholine antibody.

In terms of solving the problem underlying the invention, reference is also made to document D14. This document discloses the removal of anti-phosphorylcholine antibodies in order to improve the cellular immune responses of a patient against cancer. It would therefore seem, that the presently claimed administration of an anti-phosphorylcholine antibody may not be beneficial. As a consequence, the problem of providing a treatment of atherosclerosis may not be solved by a treatment allowable under medical conditions, since is would endanger the patient to whom the medicament is given.

In view of these reasons it is not possible to acknowledge an inventive step in the sense of Article 33.3 PCT for invention 2.

Invention 3

Document D19 discloses an increase in anti-phosphorylcholine antibodies due to atherosclerosis.
Document D11 discloses antibody IK17. This antibody detects OxLDL; a marker for atherosclerosis. Hence it is proposed for in vitro essay for the presence of atherosclerosis.
Document D20 discloses an increase in anti-phosphorylcholine IgM and IgG antibodies due to atherosclerosis.

Document D21 discloses the detection of cells expressing anti-phosphorylcholine antibody by reaction with a PC-albumin conjugate.
Document D22 discloses in example 4 a method for determining the affinity of an antibody against PC using a conjugate KLH-PC.
Document D23 discloses the role of anti-PC antibodies in atherogenesis.
From each of these documents it is clear, that the antibodies are a marker of atherosclerosis, and how their presence can be determined. More specifically, in D21 and D22, their presence is determined by using a phosphorylcholine conjugate as defined in claim 13. Therefore, invention 3 does not meet the requirements of Article 33.2 PCT for novelty.

Invention 4

Document D19 discloses an increase in anti-phosphorylcholine antibodies due to atherosclerosis.
Document D20 discloses an increase in anti-phosphorylcholine IgM and IgG antibodies due to atherosclerosis.
These documents each clearly determine the increase of anti-phosphorylcholine antibodies in atherosclerosis. These document do not explicitly mention the link with ischemic cardiovascular diseases. However, atherosclerosis is a risk factor in cardiovascular diseases well known to the skilled person. Therefore, although the presently claimed invention is formally novel over these documents, the skilled person needs no inventive skills to make the missing step.

Document D21 discloses the detection of cells expressing anti-phosphorylcholine antibody by reaction with a PC-albumin conjugate.
Document D23 discloses the role of anti-PC antibodies in atherogenesis.
These documents each clearly determine the increase of anti-phosphorylcholine antibodies. These document do not explicitly mention the link with ischemic cardiovascular diseases. However, atherosclerosis is a risk factor in cardiovascular diseases well known to the skilled person. Therefore, although the presently claimed invention is formally novel over these documents, the skilled person needs no inventive skills to make the missing step.

In view of these reasons it is not possible to acknowledge an inventive step in the sense of Article 33.3 PCT for invention 4.

Re Item VIII.

In present claims 1-16, the phosphorylcholine conjugate is only partially defined. Since this conjugate is the very basis of the presently claimed inventions, these claims do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined.
Moreover, nowhere in the present application, latex beads to which phosphorylcholine is conjugated, are prepared. Therefore, claims 7 and 14 do not meet the requirements of Article 5 PCT for sufficiency of disclosure.